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Molecular Cytogenetic Characterization of a Small, Familial Supernumerary Ring Chromosome 7 Associated With Mental Retardation and an Abnormal Phenotype

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A family is described in which a mother and two of her children were mosaic for a small supernumerary ring chromosome. As the origin of the ring chromosome could not be determined by routine cytogenetic studies, fluorescent in situ hybridization was performed, which indicated that the ring chromosome was derived from the pericentromeric region of chromosome 7. Further characterization with a YAC-probe showed the involvement of the proximal q-arm of chromosome 7. Both sibs had speech difficulties and were mildly mentally retarded whereas the mother's intelligence was at the lower end of the normal range. They all had an unusual face, characterized by a flat profile, short forehead, downslant of the palpebral fissures, high and broad nasal bridge, simply formed ears, and prognathia. This is the second report of a small supernumerary ring chromosome derived from the pericentromeric region of chromosome 7, and the described clinical phenotype differs from that delineated in the previous report. Am. J. Med. Genet. 92:147–152, 2000.

KEY WORDS: small supernumerary ring chromosome 7; familial; fluorescence in situ hybridization

INTRODUCTION

The term *supernumerary marker chromosomes* (SMCs) has been used to refer to any extra chromosomes unidentifiable by cytogenetic banding techniques [Plattner et al., 1993]. Clearly, this represents a very heterogeneous group of chromosomes with varying phenotypic consequences. The range of the phenotypic expression, from normal intelligence to severe developmental retardation, is likely to be related to the size, content in euchromatine, chromosomal origin, and degree of mosaicism.

Small SMCs are found in 0.14–0.72/1000 newborns and in 0.65–1.5/1000 prenatally ascertained cases [Blennow et al., 1994]. A large proportion of these markers is derived from acrocentric chromosomes, mostly from chromosome 15 [Blennow et al., 1994]. Part of the SMCs not derived from acrocentrics consist of pericentromeric fragments of other chromosomes and usually appear as small ring chromosomes. This subgroup is often associated with a high risk of phenotypic abnormality [Daniel et al., 1994], except for markers originating from chromosomes 1, 9, and 16 [Callen et al., 1990].

Although precise identification of marker chromosomes is crucial to predict the clinical outcome of their carriers, conventional cytogenetic techniques are frequently of limited value in the characterization of these chromosomal aberrations. Recently, however, fluorescence in situ hybridization (FISH) has proven to be a powerful method for the identification of marker chromosomes [Blennow et al., 1993; Blennow et al., 1994; Callen et al., 1992; Daniel et al., 1994; Gravholt and Friedrich, 1995; Plattner et al., 1993].

The familial transmission of a supernumerary ring chromosome with phenotypic consequences has rarely been reported. We report on the association between a small supernumerary ring chromosome derived from the proximal part of the long arm of chromosome 7 and phenotypic abnormalities in a female and two of her children. To our knowledge, this is the second report of

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a small supernumerary ring chromosome derived from chromosome 7 [Blennow et al., 1993].

CLINICAL REPORTS

Case 1

Case 1, a girl, is the first child of a 25-year-old mother and a 30-year-old father, born at 44 weeks gestation after a normal pregnancy and delivery by forceps extraction. Birth weight was 4,650 g (>97th centile). She had a bifid uvula and a midline cleft palate, which was surgically corrected at 9 months. At 4 years, she was referred to our clinic for developmental delay and an unusual face. Her motor milestones were in the normal range: She could stand at the age of 1 year and walked at 2 years. Her speech development was considerably retarded: At 4 years she only spoke a few words. On physical examination, her occipito-frontal circumference (OFC) was 50.5 cm (50th centile), weight 19 kg (75th centile), length 106.6 cm (50–90th centile). The following anomalies were noted: short forehead, flat face, high and broad nasal bridge, short nose, slight left ptosis and epicanthus, short philtrum, small mouth, and low-set, posteriorly rotated and simply formed ears with a thick helix (Fig. 1a). In addition, she had a right simian crease and genua valga. She had often been treated for middle-ear infections, but hearing tests were always normal. No other anomalies were noted on physical examination. An EEG, brain CT-scan, and roentgenographs of the spine and hands made at the age of 4 years were normal. We examined her again at the age of 10 years. Her length was 153.5 cm (90th centile), weight 48 kg (90th centile), and OFC 54.5 cm (98th centile). The facial appearance was substantially unchanged (Fig. 1b). She was attending a special school for children with speech difficulties, and her speech had markedly improved although a bad articulation was still noticeable. At 9 years, her IQ was tested with the Wechsler Intelligence Scale for Children (WISC–R). The score for verbal intelligence was 57 and for performance intelligence, 73.

Case 2

Case 2, a boy, is the younger brother of Case 1 and the last born in the sibship. He was delivered at term by cesarean section after a normal pregnancy. Birth weight was 4,250 g (97th centile). At the age of 23 months, the parents noted slow psychomotor development. The developmental screening test (Bayley Scales of Infant Development) at that time showed a delay of 6 months. We examined him at 3 years. Like his sister, his speech development was severely retarded. He only spoke a few single words with poor articulation. His length was 113 cm (>97th centile), weight 22 kg (>98th centile), and OFC 53.5 cm (98th centile). His face was similar to that of his sister, with short forehead, flat face, downward slant of the palpebral fissures, high and broad nasal bridge, short nose, short philtrum, small mouth, slight prognathia, and posteriorly angulated and simply formed ears with a thick helix (Fig. 2). He has no cleft palate or bifid uvula. No other anomalies were noted on physical examination. A CT-scan of the brain only showed a slight asymmetry of the lateral ventricles.

Case 3

Case 3 is the mother of Cases 1 and 2. She also had speech difficulties of unknown origin when she was 2 years old, which were partly resolved by speech therapy. She had no cleft palate or bifid uvula. She had attended a domestic science school, and is presently working as a housekeeper. She seems to have low performance, and her speech is difficult to understand because of bad articulation. Her length is 170 cm (50–

Fig. 1. Facial appearance of Case 1 at 4 years **(a)** and 10 years **(b)**, respectively.

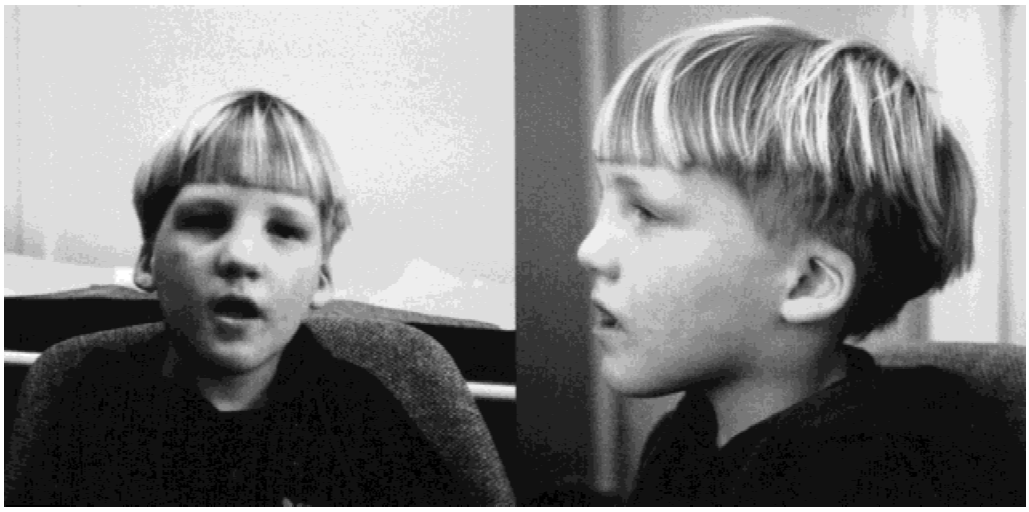

Fig. 2. Facial appearance of Case 2 at 4 years.

90th centile), weight 78 kg (>90th centile), and OFC 53.5 cm (2nd–50th centile). Facial traits similar to those of her children were present: short forehead, flat face, high and broad nasal bridge, simply formed, posteriorly angulated ears, and prognathia. The palpebral fissures were horizontal (Fig. 3). The husband and the second child are of normal intelligence and have a normal appearance.

LABORATORY STUDIES

Metaphase chromosome spreads were prepared from PHA-stimulated lymphocytes and stained by a variety of cytogenetic techniques (GTG,CBG,DA-DAPI,Ag-NOR) according to standard procedures. Fourteen metaphases of each patient were analyzed. Chromosome analysis of 59 GTG banded metaphases of the father, the healthy son, and the parents of the mother was also performed.

The origin of the marker chromosome was determined by FISH, performed as previously described [Devilee et al., 1988] using randomly chosen biotynilated probes specific for the pericentromeric region of chromosomes 7 (P7t1, GDB Id: G00–167–562), 15 (D15Z1) [Higgins et al., 1985], 18 (L1.84) [Devilee et al., 1986], and 22(p22/1:2.1) [McDermid et al., 1986]. After demonstration that the marker was derived from chromosome 7, a chromosome specific library for chromosome 7 was used. To determine whether the marker consisted of p- or q-arm, YAC-probes on proximal 7p and 7q were tested (CEPH YAC D7S520 (7q11.2); CEPH YACs D7S506, D7S494, D7S659/499 (7p11.2) [Green et al., 1994].

To investigate a deletion at 22q11 the probe was used (kindly supplied by Dr. J.H.C. Meÿers, Erasmus University, Rotterdam, The Netherlands). DNA from Patients 1 and 2 and their parents was purified from peripheral leucocytes. The parental origin of the normal chromosome 7 was determined by haplotype analysis using microsatellite markers. The polymorphic microsatellites used were D7S480 (7q31–35), D7S486 (7q31), and D7S518 (7q21-q31) [Weissenbach et al., 1992].

RESULTS

Chromosome analysis after G-banding showed, in approximately 50% of the analyzed metaphases from Cases 1, 2 and 3, a supernumerary ring chromosome smaller than a G-group chromosome (Fig. 4). C-banding of the marker showed the presence of a centromere whereas AgNOR and Da/Dapi staining were negative. FISH studies of the marker showed a negative result with the centromeric probes specific for chromosomes 15, 18, and 22, and a positive hybridization signal with the chromosome 7-specific centromeric probe (Fig. 5). Chromosome painting with a chromosome 7-specific library showed that the marker was

Fig. 3. Facial appearance of Case 3 at 37 years.

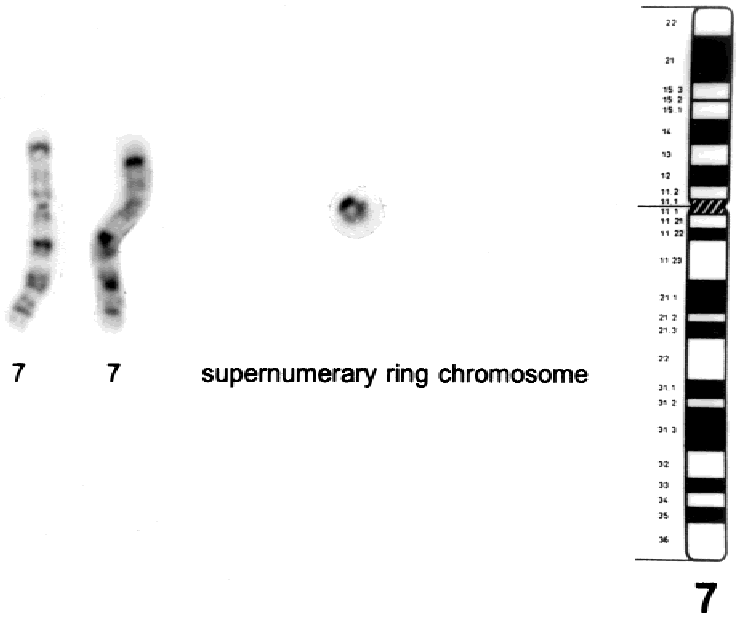

Fig. 4. Partial G-banded karyotype showing chromosomes 7 and the supernumerary ring chromosome.

entirely composed of material derived from this chromosome. With YAC probes, the ring chromosome showed a positive signal with probe D7S520 on 7q11.2 and negative results with probes on 7p11.2 (Fig. 6).

A deletion in the 22q11 region as is found in the velocardiofacial syndrome was excluded by using the M51 probe, which showed one hybridization spot on both chromosomal regions 22q11.

Chromosome analysis of the father, the healthy son, and the parents of the mother was normal. Polymorphic microsatellites on 7q showed a biparental inheritance of the normal chromosomes 7 in Cases 1 and 2.

DISCUSSION

Small supernumerary ring chromosomes can originate from the centromere and adjacent pericentromeric regions of virtually any chromosome, and are therefore composed of both noncoding heterochromatine and a

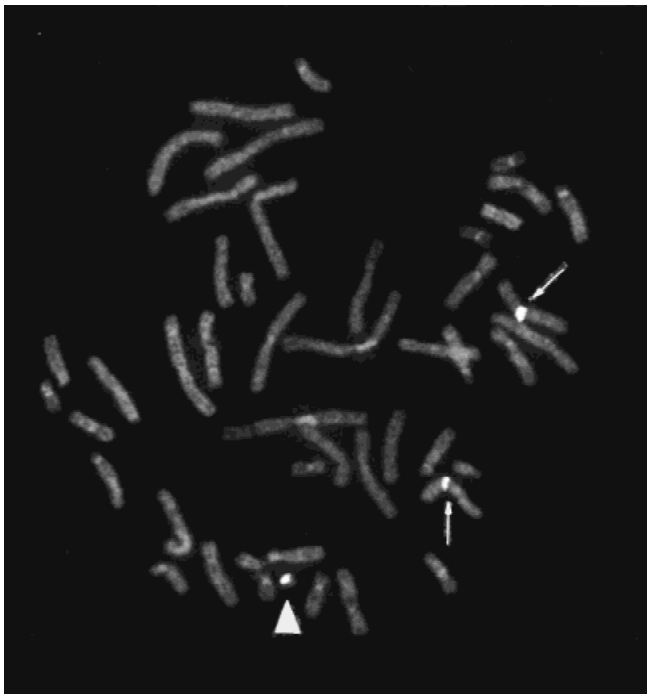

Fig. 5. Metaphase of Case 1 after FISH using a biotin-labeled α-satellite probe for the pericentro meric region of chromosome 7. Arrowhead indicates the brightly fluorescent supernumerary small ring chromosome. The two normal chromosomes 7 (arrows) also give a positive signal.

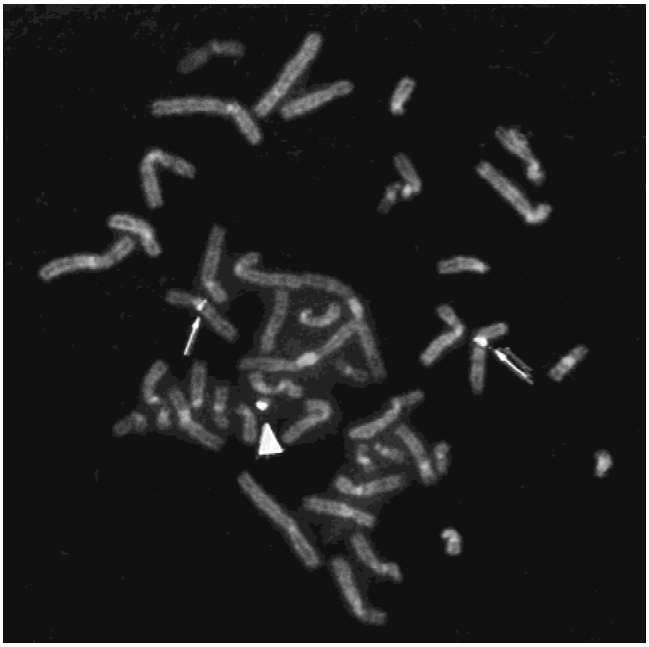

Fig. 6. Metaphase of Case 1 after FISH using a biotin-labeled α-satellite YAC probe D7S520 on the proximal q-arm of chromosome 7 (7q11.2). Arrowhead indicates the involvement of the q-arm on the small ring chromosome. The position of the probe on the proximal 7q-arm of the normal chromosomes 7 is indicated with arrows.

variable amount of pericentromeric euchromatin [Callen et al., 1991]. In the last few years, with the availability of the FISH techniques, the identification of the chromosome of origin of many markers has been made possible [Blennow et al., 1993; Blennow et al., 1995; Blennow et al., 1994; Callen et al., 1991; Callen et al., 1992; Chen et al., 1995; Daniel et al., 1994; Melnyk and Dewald, 1994; Michalski et al., 1993; Plattner et al., 1993; Rauch et al., 1992; Spinner et al., 1995].

Because this subgroup of markers will give rise to partial trisomies of the chromosome involved, the clinical consequences depend largely on the chromosomal origin and genetic content of the segments involved. According to the estimation of Daniel et al. [1994], 60% of the patients with small supernumerary ring markers are retarded with or without physical anomalies, and 20% have an unusual face without intellectual handicap.

We describe the presence of a small supernumerary ring chromosome, derived from the centromeric region of chromosome 7q, in a mother and two of her children with the following facial phenotype: flat profile, high nasal bridge, prognathia, simply formed ears, downslanting palpebral fissures (Cases 1 and 2), and midline cleft palate and bifid uvula (Case 1). The children were mildly mentally retarded and had severe speech delay. The intellectual performance of the mother was at the lower end of the normal range, and she had difficulties in speech articulation.

Although no differences were detected concerning the degree of mosaicism of the ring chromosome in lymphocytes in the mother and her children, the milder manifestations in the mother might be due to a lower percentage of abnormal cells in other tissues. The possibility that the observed clinical differences might be due to the presence of uniparental disomy for the normal chromosome 7 in the children—a situation that is probably more frequent in children of carriers of SMCs [James et al., 1995]—was excluded by the demonstration of a biparental inheritance of the normal chromosome 7 in both children.

In view of the phenotypic similarities between our cases and patients with the velocardiofacial syndrome, a microdeletion in the 22q11 region has been investigated and ruled out in our patients.

To our knowledge, only one other case of a small supernumerary ring chromosome derived from the pericentromeric region of chromosome 7 has been described [Case B; Blennow et al., 1993]. In that case, the marker was present in 100% of the lymphocytes and was paternally inherited (present in 35% of the metaphases in the father). The patient had a high and narrow forehead, downslanting palpebral fissures, low-set ears, and micrognatia. His intelligence was described as being normal but with low performance. No reference was made to speech difficulties. The father could not be clinically examined. Although the scarcity of clinical data concerning this patient hampers a comparison with our cases, an evaluation of the photographs showed a different phenotype. It is conceivable that the phenotypic differences between the patients described by us and the case reported by Blennow et al. [1993], despite the fact that in both cases the markers originated from chromosome 7, reflect differences in the amount and/or (parental) origin of chromosome 7 material [Callen et al., 1991]. A similar explanation probably accounts for the reports of different phenotypes in carriers of similar small ring markers [for review see Daniel et al., 1994].

There are reports in the literature of other cases of partial trisomies of chromosome 7 [for review see The Human Cytogenetics Database, Oxford University Press, 1994]. However, in all those instances the trisomic segments derived from chromosome 7 were considerably larger and the associated phenotypes much more severe than in our patients.

Although supernumerary marker chromosomes are usually considered to have no clinical consequences for an individual when the same marker is observed in a phenotypically normal parent, this study illustrates the possibility of intrafamilial clinical variability in carriers of apparently identical markers. Accordingly, the mother was well adapted in her social environment and had not come to medical attention before whereas a developmental delay and several physical anomalies were noted in her children. This possibility makes genetic counseling especially difficult in similar situations, particularly when such abnormalities are detected prenatally.

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